

The diagram illustrates the steps of cDNA library construction:

- Initial State:** An RNA strand (5' to 3') with a poly-A tail (AAA) at the 3' end.
- Step 1:** Addition of oligo(dT)-T₇ (100 pmol) and Reverse transcriptase. This creates an RNA-cDNA hybrid (1st strand cDNA). The RNA strand is 5' to 3' and the cDNA strand is 3' to 5' (labeled TTT-T₇).
- Step 2:** Denaturation at 99°C to separate the RNA/DNA complex.
- Step 3:** Addition of random hexamer (100 ng), Klenow fragment, and T₇ DNA Polymerase at 37°C for 2 hours. This creates a double-stranded cDNA (ds cDNA). The top strand is 3' to 5' (TTT-T₇) and the bottom strand is 5' to 3' (AAA-T₇).
- Step 4:** In Vitro Transcription using T₇ RNA Polymerase and biotinylated CTP/UTP. This produces crRNA (complementary RNA) strands (3' to 5' UUU).

FIGURE 1